National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 912a

Urea

Clinical Standard

This Standard Reference Material (SRM) 912a is certified as a chemical of known purity. It is intended primarily for calibrating apparatus and validating methods used in clinical and pathology laboratories. SRM 912a consists of 25 grams of high purity urea contained in a glass bottle. The certified values for this SRM 912a are given below.

Physical Property	wt.%	Method
Purity	99.9 ± 0.1	a.
Moisture	0.02 ± 0.003	b.
Biuret	0.02 ± 0.02	c.
Ash	0.001 ± 0.0007	d.
Insoluble Matter	0.0001 ± 0.00005	e.

a. Differential scanning calorimetry [1] indicated a purity of 99.97± 0.01 mole percent (exclusive of moisture content).

b. The moisture content was determined separately by Karl Fischer titration of samples ranging from three to ten grams.

c. Biuret was estimated spectrometrically by use of a 6 mol•L^{-1} solution of urea in aqueous, alkaline nickelous sulfate measured at the absorption maximum of biuret at 461 nm [2]. This determination was calibrated againstdilute solutions of crystalline biuret, the purity of which was established by high resolution, pulse-Fourier transform, carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy of 1 mol •L^{-1} solution of the biuret inmethyl sulfoxide-d_6.

Measured in a 2-cm cell, a 50 percent (wt/vol) solution of urea in water showed strong absorption (1.87 ± 0.01) at 220 nm, a weak absorption (0.036 ± 0.001) at 280 nm, and a very weak absorption (0.006 ± 0.001) at 461 nm.

d. The sulfated ash content was determined by volatilization of 25 g samples in porcelain crucibles, followed by moistening of the residue with concentrated sulfuric acid, further volatilization, and finally, ignition at 800 °C to constant weight.

e. Insoluble matter was determined by dissolution of 25 g samples in 100 mL aliquots of water, followed by filtration of the briefly boiled solutions through tared crucibles, which were then dried at 110 °C.

Analyses for certification of this SRM were performed in the Organic Analytical Research Division by R.G. Christensen, B.Coxon, A.L. Cummings, J. Lee, D.J. Reeder, F.J. Savluk, and L.T. Sniegoski.

The overall direction and coordination of technical measurements leading to certification were under the chairmanship of B. Coxon and D.J. Reeder.

Gaithersburg, MD 20899
December 5, 1990
(Revision of Certificate dated November 16, 1979)

William P. Reed, Acting Chief
Standard Reference Materials Program

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by R. Alvarez.

Notice and Warnings to Users:

This SRM is intended for "in vitro" diagnostic use only.

Expiration of Certification:

This certification is invalid 5 years from the date of shipping. Should it become invalid before then, purchasers will be notified by NIST.

Stability:

This material should be stored in a well-stoppered container at room temperature (30 °C or less). It should not be subjected to heat, moisture, or direct sunlight during storage. Refrigerated storage is recommended, but the material should be allowed to warm to room temperature before opening the container.

The homogeneity was verified by differential scanning calorimetry, ^{13}C NMR spectroscopy, melting point determination, and moisture determination by the Karl Fischer method. Spectroscopy by ^{13}C NMR of approximately 10 mol $\cdot L^{-1}$ solutions of the urea in H_2O:D_2O(9:1v/v), performed by signal averaging of 12,000 scans at 22.6 MHz showed only a single ^{13}C resonance with a chemical shift, δ_c, of 161.6 from external tetramethylsilane, and thus indicates the absence of organic impurities by this test.

The melting range is 133.0 to 134.0 °C, as measured in an open capillary tube heated at 0.5 °C min^{-1}.

A 10 percent (w/v) solution of this SRM in water, free from carbon dioxide, showed a pH of 7.1 ± 0.2 at 23 °C.

Preparation of Working Solutions

A standard solution containing 20 mg per 100 mL (0.2 mg per mL) of urea nitrogen may be prepared by weighing 0.429 g urea into a one-liter volumetric flask and making to volume with ammonia-free distilled water. A few drops of ACS Reagent-Grade chloroform is to be added as a preservative. The solution should be stored in a refrigerator [3]. The concentration of urea nitrogen in this solution is approximately that of the normal level in serum. An alternate procedure [4] recommends 0.1 g sodium azide per 100 mL of solution as a preservative. This standard urea nitrogen solution (20 mg/100 mL), is stable for 3 months when refrigerated at 4 °C in a well-stoppered, all-glass container. All constituted solutions of urea should be clear and without indications of bacterial growth of any kind.

References

(1) Plato, C., and Glasgow, A.R., Jr., Anal. Chem. 41, 330 (1969).

(2) Reeder, D.J., and Savluk, F.J., unpublished method.

(3) Henry, R.D., Clinical Chemistry, Principles and Practice, pp. 262-276, Hoeber Medical Division, Harper & Row, New York, N.Y. 10016 (1967).

(4) Tietz, N.W., Fundamentals of Clinical Chemistry, pp. 718-722, W.B. Saunders Co., Philadelphia, Pa. 19105 (1970).